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Early life history of the winged mapleleaf mussel (*Quadrula fragosa*)

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Abstract: Early life history information on the federally endangered winged mapleleaf (*Quadrula fragosa* (Conrad, 1835)) mussel is needed by natural resource managers to improve conservation and propagation programs. We conducted four studies to obtain some of this information. First, we observed *Q. fragosa* in the St. Croix River, Wisconsin between 1997–2010 and found females brood larvae (glochidia) for a unique period, between 8 September to 8 October at water temperatures ranging between 15–21 °C. Second, we tested 67 fish species and *Necturus maculosus* for their ability to transform glochidia into juveniles. Nearly 30,000 juvenile *Q. fragosa* were produced, but only on *Ictalurus furcatus* and *I. punctatus*. Unlike most mussel species, *Q. fragosa* glochidia grew 3 to 4-fold while attached. Third, using scanning electron microscopy we were able to distinguish *Q. fragosa* glochidia height and length from six other mussel species that also produce small glochidia (<120 µm in height). Finally, we recovered 535 juveniles from 15 naturally infested fish species to determine if any were *Q. fragosa* based on glochidia morphology. We identified one juvenile from an *I. punctatus* as a *Q. fragosa*. To improve conservation efforts we suggest monitoring not only *Q. fragosa* beds but also associated *Ictalurus* populations, whose ranges may extend beyond mussel populations.

Key words: Unionidae, freshwater mussel, brooding period, glochidia hosts

The winged mapleleaf mussel (*Quadrula fragosa* (Conrad, 1835)) was listed as a federally endangered species in 1991 as a result of range reduction, small population sizes, and poor recruitment (U.S. Fish and Wildlife Service 1991). The recovery plan for *Q. fragosa* defines recovery as the existence of at least five populations capable of long term persistence (U.S. Fish and Wildlife Service 1997). While this species was historically found in at least 34 rivers in 12 states, the only known reproducing population at the time of listing was in the St. Croix River, Wisconsin, with a possible population in the Kiamichi River, Oklahoma (U.S. Fish and Wildlife Service 1997). Since 1996, another reproducing population has been found in the Saline River, Arkansas (W. R. Posey, pers. comm.), and populations have been found in the Ouachita River, Arkansas (Posey *et al.* 1996, Serb and Harris 2003) and Bourbeuse River, Missouri (M. C. Barnhart, pers. comm.).

The findings of additional *Quadrula fragosa* populations have prompted research that has provided new insights into the biology of this species. For example, historical records describe the species as living in large streams in mud, mud-covered gravel, or gravel (Wilson and Clark 1914, Ortmann 1924, Baker 1928). Yet, *Q. fragosa* in the St. Croix River is found in areas with high water velocity in gravel, sand or rubble substrates (Hornbach *et al.* 2010). *Quadrula fragosa* is also usually found associated with dense and diverse communities of other mussels (Hornbach *et al.* 1996). These data show there is much to learn about the life history of this imperiled species.

Since most freshwater mussels require a host fish to complete their life cycle, identification of host fish species is needed by resource managers to propagate rare mussel species. For example, with knowledge of host fishes, federally listed Higgins eye (*Lampsilis higginsii* (I. Lea, 1857)) has been cultured and restocked into portions of the upper Mississippi River (Wege 2001) and several other facilities are now culturing and stocking threatened and endangered species into their historical ranges (Henley *et al.* 2001, Barnhart 2006). The lack of information on host fish species is identified as a limiting factor in many endangered freshwater mussel species recovery plans.

Host fish are usually identified either by collecting fish and identifying naturally encysted glochidia or by exposing many fish species to glochidia in the laboratory and then isolating each species until metamorphosis occurs (Hove *et al.* 2000,

Khym and Layzer 2000). Taken separately, each method has its limitations. In the wild, fish with attached glochidia may slough glochidia before metamorphosis and freshly released juveniles from these fish must be carefully scrutinized for accurate identification (Kennedy and Haag 2005, Allen *et al.* 2007). In the laboratory, metamorphosis may occur on some species that may not naturally come in contact with glochidia. Early screening efforts at the University of Minnesota (UMN) identified channel catfish (*Ictalurus punctatus*) as a suitable *Quadrula fragosa* host (Hove *et al.* 2002).

Development of embryonic and encapsulated glochidia is thought to be temperature-dependent. For example, the development of embryonic duck mussel (*Anodonta anatina* (Linnaeus, 1758)) glochidia was influenced by temperature, brooding glochidia at the time of highest water temperature – presumably because this results in a high output of glochidia at a low energetic cost (Jokela *et al.* 1991). Higher water temperature generally shortens the period of metamorphosis (Roberts and Barnhart 1998, Gray *et al.* 2002). The time required for metamorphosis of *Quadrula fragosa* glochidia is predictable based on water temperature (Steingraeber *et al.* 2007). However, the amount of time glochidia are brooded in the marsupial gills is also important in regulating the length of time to metamorphosis (Khym and Layzer 2000).

Since an understanding of the early life history of *Quadrula fragosa* is crucial for propagation efforts to protect and restore this species, the objectives of this study were to: (1) describe *Q. fragosa* brooding period, (2) determine suitable hosts for *Q. fragosa* glochidia, (3) compare the morphology of *Q. fragosa* glochidia with other mussel species in the St. Croix River, and (4) collect juvenile *Q. fragosa* from naturally infested fishes.

MATERIALS AND METHODS

Brooding behavior

We studied *Quadrula fragosa* brooding behaviors in the St. Croix River and at the UMN's Wet Laboratory between 1997–2010. Between May to November 1997 and April to October 1998 we collected 6–25 mussels bimonthly during daylight hours using SCUBA and snorkel equipment and checked them for gravidity. Gravid animals were identified as those that had gills swollen with embryos, glochidia or conglutinates (glochidia aggregations within a gelatinous matrix) or held conglutinates beneath a mantle magazine (Barnhart *et al.* 2008). We also observed mussels approximately biweekly between September to October 2000–2010 while we gathered brooding females for U.S. Fish and Wildlife Service (USFWS) juvenile propagation efforts. During this time we increased the probability of finding *Q. fragosa* by creating several aggregations of 10–15 adults in mid-summer before the suspected reproductive period in August. Between 2000–2010 we terminated searches for brooding mussels when the propagation quota was met so the end of the brooding period was likely missed during many years. We identified mussels using Sietman (2003) and estimated mussel age by counting external annuli.

Host suitability trials

To initiate host suitability trials we obtained glochidia from brooding *Quadrula fragosa* from the St. Croix River, Minnesota. These mussels were held 1–2 wk in 0.5–1 L beakers placed in either flow-through or static 40 L aerated aquaria containing well water at temperatures ± 2 °C of river temperatures. The methods used to examine glochidia are described in Hove *et al.* (2011). Viable glochidia were exposed to potential hosts within 12 h of release. We estimated the number of glochidia used for some exposures by placing glochidia in a flask containing a known volume of water and suspending the contents. From this slurry we quickly removed a 2 mL aliquot with a pipette and preserved it with 70% ethanol. These glochidia were later enumerated using a dissection microscope and cross polarized light. For most trials we used glochidia from a single female but glochidia from up to three females were combined and exposed to some fish species. Due to limited numbers of brooding females, we could not run replicate trials with all fishes from various sources under different thermal regimes. We calculated the percentage of metamorphosed glochidia = (no. juveniles recovered/no. glochidia recovered x 100) for some host trials.

We followed standard protocols (Zale and Neves 1982, Hove *et al.* 2000) to determine suitable hosts and obtain juvenile mussels from naturally infested fishes. To obtain potential hosts we primarily collected them from central and southern Minnesota, or they were provided by private, state, or federal hatcheries. These animals were held for > 2 months before use in host suitability trials, which we initiated in September to October 1998–2000 and 2002–2003. Generally, we used standard methods described in Hove *et al.* (2011) to infest fish with glochidia. A small number of fishes (flathead catfish (*Pylodictus olivaris*) and slender madtom (*Noturus exilis*) were anesthetized with Finquel® (tricaine methanesulfonate) and glochidia were pipetted directly onto their gills.

Host suitability trials were conducted under two thermal regimes. We conducted most host suitability trials at the lowest temperature available at our UMN laboratory (11 °C) to mimic water temperatures encountered by this species during glochidia release in October (10 ± 3 °C). However, we had to maintain fish for 6–9 months at this temperature to facilitate glochidia metamorphosis and the lower temperatures presumably facilitated several outbreaks of "Ich" (*Ichthyophthirius multifilis*) in which many *Ictalurus punctatus* died We used a combination of wild and hatchery-reared fishes and tested

67 fish species and the mudpuppy [a salamander] (*Necturus maculosus*) in the low temperature trials. We conducted trials at warmer temperatures (19–20 °C) at the Upper Midwest Environmental Sciences Center (UMESC) to increase the likelihood of *I. punctatus* survival, rapidly test different species of host fish candidates, and mimic temperature encysted *Quadrula fragosa* might experience during September in the St. Croix River (18 ± 3 °C). We used hatchery-reared blue catfish (*I. furcatus*) and channel catfish, as well as wild tadpole madtom (*Noturus gyrinus*), *N. exilis*, and *Pylodictus olivaris* for the warm water trials.

We held animals with attached glochidia in aquaria until glochidia were sloughed or juvenile mussels were released. Depending on the size of fish we held animals at a density of 1–17/aquarium. We fed fish held at 11 °C 1–2 times per wk and those at 15–22 °C 3 times per wk. Small fish (*e.g.* cyprinids, etheostomids, catostomids) were fed frozen brine shrimp (*Artemia* sp.) or blood worms (larval chironomids) and piscivorous fish fathead minnows (*Pimephales promelas*). At the UMESC we conducted host suitability trials similarly, except that test fish were held in 38 L aquaria fitted with false bottoms (plastic screen with 25 mm^2 openings) positioned 3.5 cm above the aquarium floor and were fed small rainbow trout (*Oncorhynchus mykiss*) once per wk.

We followed methods described in Hove *et al.* (2011) to sieve siphonate through a 45 μm sieve, search for glochidia, identify juveniles, and terminate host suitability trials. Unionid, fish, and amphibian nomenclature follows Turgeon *et al.* (1998), Robins *et al.* (1991), and Crother *et al.* (2000), respectively.

Glochidia morphology

To help us identify juvenile mussels released from naturally infested fishes we collected glochidia from seven St. Croix River mussel species that produce small glochidia (height <120 μm) (spectaclecase (*Cumberlandia monodonta* (Say, 1829)), fragile papershell (*Leptodea fragilis* (Rafinesque, 1820)), *Quadrula fragosa*, mapleleaf (*Q. quadrula* (Rafinesque, 1820)), pistolgrip (*Tritogonia verrucosa* (Rafinesque, 1820)), fawnsfoot (*Truncilla donaciformis* (I. Lea, 1828)), and deertoe (*T. truncata* Rafinesque, 1820) (Surber 1912, 1915). These glochidia were obtained from gravid females from the St. Croix and Mississippi rivers, Minnesota and preserved in 95% ethanol. We washed glochidia with 100% ethanol and mounted them on conductive tape on scanning electron microscope specimen stubs. To coat specimens with gold we used a Fullam Sputter Coater and then viewed them with a Hitachi S3500N variable pressure scanning electron microscope with Quartz PCI digital imaging software (Quartz Imaging Corporation, Vancouver, British Columbia, Canada). To measure valve dimensions (Hoggarth 1999) we printed photomicrographs of glochidia and measured valve dimensions to the nearest mm with a ruler. We converted measures to microns using an electronically-generated scale bar printed with each image. To measure growth of freshly-released juvenile *Q. fragosa* recovered from *Ictalurus punctatus* trials at the UMESC we used a dissecting microscope and ocular micrometer. We analyzed differences in valve dimensions among species using nested ANOVA with length or height as the dependent variable, species as the major independent factor, and individual female as the minor factor. Multiple means comparison tests (Tukey's *HSD*) were also conducted (JMP v. 5, SAS Institute, Cary, North Carolina).

Natural infestations

We collected fishes naturally infested with glochidia around mussel beds in the St. Croix River that contained *Quadrula fragosa*. We captured fishes between April to June and September to October, 1998–2005, and visually inspected them for glochidia. Infested fishes were held in flow through aquaria (40–500 L) containing well water adjusted between 11–23 °C to follow the change in St. Croix River water temperature measured at the USGS gauging station, St. Croix Falls, Wisconsin until juveniles released or glochidia were sloughed. To recover juveniles we used the procedures described above for host suitability trials.

To aid in identification of a juvenile mussel recovered from a naturally infested *Ictalurus punctatus* we also conducted host suitability trials. We exposed *I. punctatus* to glochidia from *Cumberlandia monodonta*, *Leptodea fragilis*, *Tritogonia verrucosa*, and *Truncilla truncata* during 1996–2006 using methods previously described at water temperatures between 16–20 °C. We assumed that negative results in these trials would provide additional evidence that the wild juvenile we recovered was either a *Quadrula fragosa* or *Q. quadrula*.

RESULTS

Brooding behavior

Quadrula fragosa females from the St. Croix River brooded young in all four gills during the fall. Before *Q. fragosa* began brooding some individuals, presumably females, began to emerge from the substrate as early as mid-August. We noted an increase in the incidence of emergence prior to and during the brooding period. Gravid females with swollen gills or a mantle magazine were observed once (24 September) in 1997. In 1998 we observed females brooding between 10 September to 8 October at 15–21 °C with the percentage of gravid females peaking at 58% on 24 September (Fig. 1). Between 2000–2010 we observed brooding animals between 8–29 September. The youngest and oldest gravid females we observed were 8 and 18 y, respectively.

Brooding *Quadrula fragosa* exhibited several characteristics useful to propagation efforts. Brooding females were

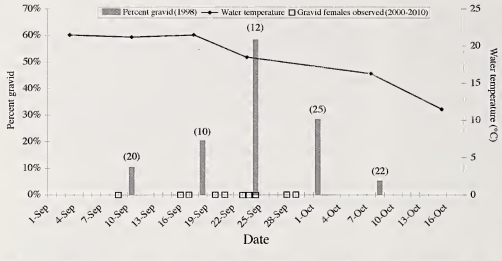

Figure 1. Brooding period of *Quadrula fragosa* in the St. Croix River during 1998. Numbers in parentheses are the number of females checked for gravidity. The black line denotes water temperatures and the unfilled squares denote dates gravid *Quadrula fragosa* were observed during 2000–2010.

relatively obvious as they often had half or more of their valves raised above the river bed, and frequently presented a swollen, gray, crenulated mantle magazine just dorsal to the incurrent aperture (Fig. 2). We usually selected females with fully developed displays when seeking mature glochidia. Since brooding females sometimes released glochidia when removed from the substrate, divers placed them in separate sealed plastic bags before bringing them onto the boat. To determine if a non-displaying mussel was brooding glochidia, we opened the valves slightly and illuminated the gills for examination. Gills bearing glochidia were only 2–3 fold thicker than non-brooding gills. Gills bearing glochidia often had fine white lines, ~1 mm wide, oriented 45–90° to gill filaments. These lines often consisted of a series of small white dots that contrasted in color with the yellowish-white gills, and were presumably conglutinates.

Gravid *Quadrula fragosa* held conglutinates and glochidia in their marsupia or within the magazine. *Quadrula fragosa* released glochidia in whole and broken conglutinates and individually in the laboratory (Fig. 3). Conglutinates were white in color, had an elongated rectangular outline that often tapered to a rounded point and some conglutinates were joined at one end. Whole conglutinates were 50–120 mm long and 2–3 mm wide.

Figure 2. Image of gravid *Quadrula fragosa* in the St. Croix River on 17 September 2010; **A**, posterior view and **B**, lateral view. Scale bars = 1 cm. Photo credit to Nick Rowse, U.S. Fish and Wildlife Service.

Host suitability trials

More than 28,000 juvenile *Quadrula fragosa* were recovered during host suitability trials. Of the 67 fish species (in 20 families) and *Necturus maculosus* exposed to *Q. fragosa* glochidia, only *Ictalurus furcatus* and *I. punctatus* facilitated metamorphosis (Table 1). *Ictalurus furcatus* appeared to facilitate metamorphosis of a greater proportion of glochidia than *I. punctatus* where a similar number of glochidia were exposed to fishes. However, females contributing glochidia and glochidia exposure times varied between trials, and our small number of replicate trials prevented statistical comparison of juvenile production between fish species. *Ictalurus furcatus* maintained at 20 °C produced several thousand juveniles after 20–50 d of attachment (Table 1, trials 2–5). In contrast, *I. furcatus* maintained at 11 °C took 70–230 d to produce juveniles (Table 1, trial 1). The data for *I. punctatus* were considerably more variable, but in general, metamorphosis took longer at 11 °C than at warmer temperatures (Table 1, compare trials 7 and 18). The length of attachment periods was similar between *I. furcatus* and *I. punctatus* at 20 °C (Table 1, compare trials 2–5 to trials 14–17). We did not obtain enough glochidia to have replicate tests within a given fish source, so we cannot address this potential source of variation in our trials.

Survival of host fish was 100% in the flow through systems used at the UMESC. No disease outbreaks occurred presumably due to higher test temperatures that facilitated metamorphosis during a brief period (~6 wk). Survival of host fish was considerably more

Figure 3. Conglutinates and glochidia of *Quadrula fragosa*.

variable at UMN, presumably due to *Ichthyophthirius multifilis* outbreaks among *Ictalurus punctatus* during the prolonged periods of metamorphosis at cooler temperatures. These data illustrate the importance of maintaining potential host fish health before and during testing. However, even though fish health problems were not encountered at the UMESC, the number of juveniles produced in trials using the same fish species (from the same source) and conducted at the same test temperature varied 500-fold (Table 1, trials 14–17). This variation is likely attributed to the fact that we did not quantify the number of glochidia exposed to fish and because different females were used in these trials (Table 1).

Glochidia morphology

Quadrula fragosa glochidia were readily distinguished from the glochidia of 6 other St. Croix River mussel species that also produce small glochidia (Fig. 5). Glochidial valve height and length varied significantly among species ($F_{6, 195}$ = 1660, $P < 0.0001$ and $F_{6, 198}$ = 1770, $P < 0.0001$, respectively). Glochidia height was significantly different between all species except for *Truncilla truncata* vs. *T. donaciformis*, and *T. donaciformis* vs. *Cumberlandia monodonta*, and glochidia length was significantly different between all species except for *T. truncata* and *T. donaciformis* (Table 2). In addition, glochidia from *C. monodonta* were more spherical than the other species and glochidia from *Leptodea fragilis* had a pitted surface. There were significant differences in glochidia height and length among females within species ($F_{32, 195}$ = 13.8, $P <$ 0.0001 and $F_{32, 198}$ = 7.1, $P < 0.0001$, respectively).

Quadrula fragosa grew during attachment in some trials. *Quadrula fragosa* glochidia had a subelliptical valve shape, short dorsal alae, and exterior surface sparsely-malleated dorsally. Glochidia valve height averaged 102 ± 2 µm and 86 ± 3 µm in length (n = 24, ± 1 *SD*). Newly-released juveniles (261–263 d post-exposure on *Ictalurus punctatus* at 0–21 °C) averaged 340 ± 3 µm in height and 370 ± 4 µm in length (n = 35). These changes represent about a 3-fold increase in the height and a 4-fold increase in the length of valves while attached to the host fishes (Fig. 4). *Quadrula fragosa* glochidia also increased up to two-fold in length while attached to black bullhead (*Ameiurus melas*), brown bullhead (*A. nebulosus*), yellow bullhead (*A. natalis*), *Noturus exilis*, and flathead catfish, although metamorphosis was not observed on these, or other species tested (Table 1). *Quadrula fragosa* glochidia did not increase in size during attachment to any other potential host species.

Natural infestations

We collected 689 fishes representing 43 species in 17 families from the St. Croix River and recovered 535 juvenile mussels from 15 species. Of the juveniles we recovered, only one (from an *Ictalurus punctatus*) had a glochidial valve height (101 µm), length (88 µm), and outline similar to *Quadrula fragosa*. The only mussel species whose observed range in glochidia size encompassed these measurements was *Q. fragosa*. In host suitability trials, *I. punctatus* sloughed glochidia from *Cumberlandia monodonta* (number of trials, mean number of fish per trial; 3, 4), *Leptodea fragilis* (1, 5), *Tritogonia verrucosa* (5, 5), and *Truncilla truncata* (1, 4) in < 12 d at 17 ± 3 °C, providing supporting evidence that the juvenile recovered from the naturally infested *I. punctatus* was a *Q. fragosa* or *Q. quadrula*.

DISCUSSION

The period when *Quadrula fragosa* broods its young appears to be unique. Most *Quadrula* species (10 of 13 species researched) brood glochidia between May to August although two species begin brooding in April and one has been observed brooding in January in Texas (Howard 1914, Utterback 1915, Ortmann 1919, Baker 1928, Yeager and Neves 1986, Yeager and Saylor 1995, Howells *et al.* 1996, Howells 2000, Haag and Warren 2003). To our knowledge, *Q. fragosa* is the only *Quadrula* that has been observed brooding in September, and in fact, no other Mississippi River basin mussel species has been reported brooding glochidia briefly in the fall. Thorough brooding period studies are needed for many North American freshwater mussels (Parmalee and Bogan 1998, Williams *et al.* 2008). Future studies will reveal whether or not the brief fall *Q. fragosa* brooding period is indeed truly unique.

We conducted host suitability trials on 67 potential host fishes in 20 families, representing 72% of fish species (79% of fish families) native to mid- and lower reaches of the St. Croix

Table 1. Fish species that facilitated metamorphosis of *Quadrula fragosa* glochidia into juveniles in the laboratory. Sources of host fish were: SCR-Saint Croix River, Minnesota or Wisconsin; CROW-Crow River in Minnesota; MSR-Mississippi River, Minnesota; Minnesota DNR-Minnesota broodstock, raised in a Minnesota Department of Natural Resources hatchery; and USGS-*Ictalurus punctatus* from Senecaville State Fish Hatchery, Ohio, and *I. furcatus* from Normandy Fish Hatchery, Tennessee. "Individual female(s) used for exposure" column lists identification numbers.

Trial	Host fish source	Testing facility	Individual female(s) used for exposure	Mean daily temperature[a] (°C)	Length (mm)	No. fish exposed	Estimated no. glochidia exposed to fish	No. surviving fish	Release period in days[b]	No. live juveniles recovered	Percentage of meta-morphosed glochidia
					Ictalurus furcatus						
1	USGS	UMN	3, 4, 5	11	160–200	6	[c]	2[d]	71–230	2182	[c]
2	USGS	UMESC	2	20	185–210	5	2120	5	30–49	477	19
3	USGS	UMESC	2	20	163–213	5	18960	5	27–49	3974	40
4	USGS	UMESC	5	20	168–194	5	70200	5	26–49	4283	72
5	USGS	UMESC	7	20	178–211	5	24325	5	21–49	2222	50
6	USGS	UMN	1, 2	22	180–260	7	[c]	7	28–56	813	[c]
					Ictalurus punctatus						
7	CROW	UMN	1, 2, 5	11	60–80	15	[c]	8	61–88	9	[c]
8	MSR	UMN	1, 2, 5	11	210–320	5	[c]	1	88–225	743	[c]
9	SCR	UMN	8	11	300	1	[c]	0	117–123	2	[c]
10	SCR	UMN	3	11	210–360	3	[c]	2	(33–38)	0	[c]
11	USGS	UMN	9	11	200–300	4	[c]	0	139–260	252	[c]
12	USGS	UMESC	5	[0–21]	237–263	7	> 87750	7	243–270	3450	[c]
13	USGS	UMESC	5	[13–20]	263–300	5	> 87750	5	60–89	9337	[c]
14	USGS	UMESC	5	20	137–156	5	70200	5	26–40	533	14
15	USGS	UMESC	2	20	129–143	5	2120	5	36	1	0.1
16	USGS	UMESC	2	20	125–146	5	18960	5	28–40	18	1
17	USGS	UMESC	7	20	133–161	5	24325	5	23–37	49	2
18	CROW	UMN	3	22	50–80	20	[c]	12	34–38	1	[c]
19	MN DNR	UMN	2	22	200–300	3	[c]	3	(9–14)	0	[c]
20	MSR	UMN	3	22	230–300	3	[c]	3	(34–38)	0	[c]
21	SCR	UMN	1, 2	22	300	3	[c]	1	(14–24)	0	[c]

[a] Bracketed data are mean daily temperature ranges for trials with thermal regimes that varied > 2 °C

[b] Data in parentheses are glochidia attachment periods where metamorphosis was not observed

[c] Number not estimated

[d] All 6 fish were alive up to day 216

Fish species (number of trials, mean number of fish per trial, range of days to rejection) that did not facilitate glochidia metamorphosis at 11°C unless noted otherwise (* some or all trials at 19–21°C): (1) Native fishes-*Acipenser fulvescens* (2, 3, 1–6), *Scaphirhynchus platorynchus* (1, 2, 1–2), *Lepisosteus osseus* (1, 4, 1–4)*, *Lepisosteus platostomus* (1, 4, 1–4)*, *Amia calva* (1, 2, 1–2), *Anguilla rostrata* (1, 2, 5)*, *Campostoma anomalum* (2, 8, 8–11), *Carassius auratus* (1, 1, 1–3), *Cyprinella spiloptera* (3, 9, 1–4), *Luxilus cornutus* (2, 8, 1–4), *Nocomis biguttatus* (3, 6, 1–4), *Notemigonus crysoleucas* (2, 2, 1–4)*, *Notropis atherinoides* (2, 11, 1–3), *Notropis blennius* (2, 6, 1–3), *Notropis dorsalis* (1, 22, 1–3), *Notropis hudsonius* (1, 6, 1–4), *Notropis stramineus* (2, 12, 1–4), *Phoxinus eos* (1, 19, 1–2), *Pimephales notatus* (3, 11, 1–4), *Pimephales promelas* (3, 13, 1–4), *Rhinichthys cataractae* (3, 14, 7–15), *Semotilus atromaculatus* (2, 2, 2–5), *Catostomus commersoni* (1, 14, 1–3), *Hypentelium nigricans* (2, 1, 1–4)*, *Moxostoma macrolepidotum* (1, 22, 1–3), *Ameiurus melas* (5, 11, 20–120)[f], *Ameiurus natalis* (5, 9, 8–118)[f], *Ameiurus nebulosus* (1, 4, 25–27)[f], *Noturus exilis* (8, 3, 14–95)*[f], *Noturus flavus* (2, 2, 1–6), *Noturus gyrinus* (4, 8, 4–15), *Pylodictus olivaris* (8, 3, 8–82)*[f], *Esox lucius* (1, 8, 2–8), *Umbra limi* (2, 5, 6–15), *Percopsis omiscomaycus* (1, 1, 1–4)*, *Lota lota* (3, 2, 2–11), *Fundulus diaphanus* (1, 2, 1–4)*, *Culaea inconstans* (3, 3, 22–26), *Cottus bairdii* (2, 7, 2–8), *Cottus cognatus* (1, 13, 2–8), *Morone chrysops* (1, 5, 1–4)*, *Ambloplites rupestris* (1, 17, 1–2), *Lepomis cyanellus* (3, 12, 15–21), *Lepomis gibbosus* (1, 4, 1–2), *Lepomis humilis* (2, 2, 1–4), *Lepomis macrochirus* (4, 4, 7–15), *Micropterus dolomieu* (1, 12, 1–2), *Micropterus salmoides* (2, 11, 6–15), *Pomoxis nigromaculatus* (3, 5, 6–15), *Ammocrypta clara* (1, 21, 1–3), *Crystallaria asprella* (1, 2, 1–3), *Etheostoma exile* (2, 7, 4–7), *Etheostoma caeruleum* (2, 9, 7–15), *Etheostoma flabellare* (1, 14, 1–4), *Etheostoma nigrum* (4, 16, 4–7), *Perca flavescens* (2, 28, 3–7), *Percina caprodes* (3, 8, 3–7), *Percina maculata* (1, 24, 4–7), *Percina phoxocephala* (2, 3, 2–5), *Percina shumardi* (1, 4, 1–4), *Sander canadensis* (1, 4, 1–2), *Sander vitreus* (1, 10, 1–2), *Aplodinotus grunniens* (2, 3, 4–9)*, (2) Non-native fishes-*Oncorhynchus mykiss* (1, 1, 1–4)*, *Poecilia reticulata* (2, 4, 13–15), (3) Amphibians-*Necturus maculosus* (1, 2, 1–2).

[e] Attachment period unclear

[f] Glochidia grew during some trials.

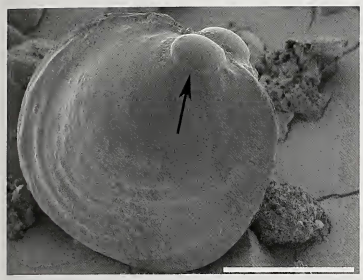

Figure 4. Newly-released juvenile *Quadrula fragosa* recovered from an *Ictalurus punctatus* held at 11°C. Note the glochidia valves (indicated by an arrow) located along the hinge of the juvenile valves. Scale bar = 200 µm.

River (Becker 1983, Fago 1986), and only *Ictalurus furcatus* and *I. punctatus* facilitated glochidia metamorphosis. This is the first time *I. furcatus* has been shown to facilitate metamorphosis of *Quadrula fragosa* under laboratory conditions. In rivers with extant *Q. fragosa* populations *I. punctatus* occurs in mid- and lower river reaches of the Bourbeuse, Kiamichi, Ouachita, St. Croix, and Saline rivers. Meanwhile, *I. furcatus* is less abundant in the downstream reaches of these rivers except for the St. Croix and Bourbeuse rivers where they do not occur (R. L. Benjamin, J. C. Boxrucker, S. E. McMurray, J. A. Olive, J. W. Quinn and D. B. Zapitello, pers. comm.). Given that *I. furcatus* has not been reported from the St. Croix River since they were stocked in 1977 (Phillips *et al.* 1982) and juvenile *Q. fragosa* have recently been found in the St. Croix River (D. J. Hornbach and M. C. Hove, pers. obs.), the absence of *I. furcatus* does not likely limit this *Q. fragosa* population. A large *I. punctatus* population does exist in the St. Croix River (Fago 1986). Future research efforts should include determining natural infestation rates of *I. furcatus* and *I. punctatus* in other rivers, comparing juvenile production rates between suitable host species, and determining host suitability of members of at least four fish families not analyzed in this study (*e.g.* Petromyzontidae, Hiodontidae, Clupeidae, Atherinopsidae).

Quadrula fragosa has among the most specific host requirements of any mussel species. A single host species has been suggested for only a few mussel species (Watters 1994), although this may be due to limited study. Many unionid species will metamorphose on several suitable hosts (cf., Watters 1994, 2010). The fact that *Ictalurus punctatus* is currently common and widely-distributed in the Mississippi River basin suggests that although *Q. fragosa* has very specific host requirements, its endangered status is probably due to factors other than limited host abundance.

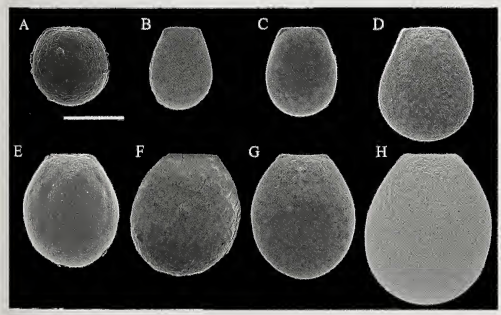

Figure 5. Scanning electron micrographs of glochidia from unionid mussels in the St. Croix River that release small glochidia (scale bar = 50 µm). Species are: **A**, *Cumberlandia monodonta*, **B**, *Truncilla donaciformis*, **C**, *T. truncata*, **D**, *Leptodea fragilis*, **E**, *Quadrula quadrula*, **F**, glochidial valve (100 µm in valve height) from a juvenile mussel recovered from a naturally infested *Ictalurus punctatus*, **G**, *Quadrula fragosa*, and **H**, *Tritogonia verrucosa*.

The rate of glochidia metamorphosis for *Quadrula fragosa* appears to be temperature-dependent, with metamorphosis occurring more rapidly at warmer temperature. This supports earlier research that showed the rate of metamorphosis for *Q. fragosa* ranged from 28–37 d in a constant (19 °C) thermal regime, totaled 70 d in a varied (12–19 °C) thermal regime, and ranged from 260–262 d in simulated natural (0–21 °C) thermal regimes (Steingraeber *et al.* 2007). These data indicate that St. Croix River *Q. fragosa* infesting a host early in the brooding period could metamorphose during a warm fall and those attaching later would likely over-winter on the host. The positive relationship between metamorphosis rate and temperature is well established in other mussel species (Roberts and Barnhart 1998, Araujo *et al.* 2002, Gray *et al.*

Table 2. Mean glochidia valve heights and lengths, standard deviations and ranges observed for St. Croix River mussel species that produce small glochidia (*i.e.* valve height < 120 μm). Valve heights and lengths with different superscripts were significantly different ($P < 0.050$).

Species	No. females	No. of glochidia measured	Mean valve height (μm ± 1 SD)	Height range	Mean valve length (μm ± 1 SD)	Length range
Cumberlandia monodonta	8	39	61 ± 3^{a}	55–67	61 ± 2^{a}	57–64
Truncilla donaciformis	3	16	$61 \pm 2^{a,b}$	56–64	47 ± 3^{b}	42–52
Truncilla truncata	4	21	66 ± 4^{b}	58–72	52 ± 3^{b}	46–56
Leptodea fragilis	6	23	89 ± 11^{c}	57–99	76 ± 6^{c}	59–82
Quadrula quadrula	2	19	92 ± 4^{d}	83–97	84 ± 3^{d}	80–91
Quadrula fragosa	5	24	102 ± 2^{e}	96–107	86 ± 3^{e}	82–92
Tritogonia verrucosa	11	57	119 ± 6^{f}	108–132	102 ± 4^{f}	92–111

2002). Even though these data show that warmer culture temperatures can produce juveniles in shorter periods of time, at lower cost, and with fewer losses of host fish, potential pitfalls warrant caution in adopting this approach to propagation. In the flat floater (*Anodonta suborbiculata* Say, 1831), a species that releases glochidia in the winter, the percentage of glochidia that metamorphosed was inversely related to water temperature (Roberts and Barnhart 1998). Juvenile *Q. fragosa* that metamorphosed over 8 to 9 months under conditions mimicking seasonal variations in the St. Croix River (0–21 °C) had valve morphology characteristic of the species, while juveniles that metamorphosed after 2 months under unseasonably warm temperatures (12–20 °C) were atypical (Steingraeber *et al.* 2007). M. C. Barnhart (pers. comm.) observed *Q. fragosa* juveniles release earlier at 23 °C but they were smaller than those held through the winter at lower temperatures. These findings indicate the need to carefully evaluate many factors that may influence glochidial metamorphosis to ensure not only high levels of viability, but also expression of natural genetic traits among propagated juveniles.

Culture of juvenile *Quadrula fragosa* should follow the natural thermal regime. It has been suggested that the parasitic stage of mussels serves three functions: (1) metamorphosis, (2) timing of excystment to minimize mortality in the first y, and (3) dispersal (Jokela *et al.* 1991). Even though *Q. fragosa* can be cultured quickly in warm water and restocked in late fall (function 1), this action may increase overwintering mortality of juveniles (function 2). Overwinter mortality may be decreased through two mechanisms. First, juveniles can be grown to a size that is less susceptible to predation in the wild (Beaty and Neves 2004). Second, the quantity and quality of food resources available during winter may not be adequate for survival. Food resources in the upper Mississippi River vary seasonally with diatoms dominating in the spring and green algae becoming more abundant during late spring (Baker and Baker 1981, Huff 1986, Lutteton *et al.* 1986). Although we know very little about the nutritional requirements of juveniles, green algae and diatoms appear to be important in their diets, at least in laboratory culture (Gatenby *et al.* 1996, 1997). In the marine mussels, the timing of larval food has been shown to be important for performance of juveniles (Phillips 2004). Thus, we suggest culturing juvenile *Q. fragosa* under a natural thermal regime until there is a greater understanding of the factors influencing juvenile survival.

Glochidia growth while encysted occurs in few mussel species. Glochidia of pimpleback (*Quadrula pustulosa pustulosa* (I. Lea, 1831)), *Q. quadrula* and *Tritogonia verrucosa* are all reported to grow while encysted on ictalurids (Howard 1913, Howard and Anson 1922, Pepi and Hove 1997). In addition to *Quadrula*, growth of encysted glochidia is reported in only two other mussel lineages: *Margaritifera* spp. (Nezlin *et al.* 1994), and the Lampsiline clade including *Potamilus* Rafinesque, 1818 and *Truncilla* Rafinesque, 1819 (Surber 1913, Utterback 1916, Coker *et al.* 1921). Perhaps selective pressures exist for juvenile mussels to reach a minimum size (e.g. 300–400 μm) or shape (e.g. discoidal) before they release from their hosts. For example, anterior-posterior valve growth closes the large gap between glochidial valves in *Potamilus* spp. thereby improving the defensive roll of the shell. Or perhaps a larger size is needed for juvenile mussels to settle in appropriate habitat after leaving their host (Barnhart *et al.* 2008). Additional work is needed to determine whether glochidial growth occurs among other mussel species, especially those that release small glochidia, and to fully elucidate the adaptive significance of this trait.

Several facts support our identification of the juvenile mussel recovered from the naturally infested *Ictalurus punctatus* as a *Quadrula fragosa*. First, glochidia valve characters were consistent with previous studies (*e.g.* Hoggarth 1999). Second, *Q. fragosa* metamorphose on *I. punctatus*, but many St. Croix River mussel species with small glochidia (*Cumberlandia monodonta*, *Leptodea fragilis*, *Tritogonia verrucosa*, and *Truncilla*

truncata) do not (Howells 1997). Another St. Croix River species that produces small glochidia is *Q. quadrula*. This species broods glochidia during April to August (Howard 1914, Coker *et al.* 1921), and grow and metamorphose on *I. punctatus* between 51–68 d at rising water temperature from 13–20 °C (Schwebach *et al.* 2002). Juvenile *Q. quadrula* and *Q. fragosa* are probably released naturally in the St. Croix River during May and June, and we recovered the juvenile described in this study during June from a fish caught mid-May. However, the glochidial valve height of the recovered juvenile was greater than the range observed for *Q. quadrula* glochidia. Third, *Q. quadrula* are very rare in the reach of the St. Croix River where *Q. fragosa* occur. No *Q. quadrula* were observed among 4043 mussels collected during 10 y of sampling in St. Croix River's primary *Q. fragosa* bed, yet *Q. fragosa* consisted of 0.1% of the mussel community (Hornbach 2001). Collectively, these data lead us to conclude that the juvenile mussel recovered from the naturally infested *I. punctatus* was a *Q. fragosa*.

Conservation of *Quadrula fragosa* will partly depend on meeting the species' early life history needs. We have shown that *Ictalurus punctatus* and *I. furcatus* are likely hosts in the wild and can be used to successfully propagate *Q. fragosa* in captivity. Although propagation is more rapid at warmer temperatures, the thermal environment in which juveniles are restocked needs to be considered in any propagation program. It is unlikely that the decline of *Q. fragosa* is currently limited by host fish, as *I. punctatus* is relatively abundant in the St. Croix River. However, given that there is a small commercial fishery for *I. punctatus* in the St. Croix River and that *I. punctatus* probably overwinter in Lake St. Croix near the confluence with the Mississippi River where commercial fish harvest is greater (J. Gordon and D. B. Zapitello, Minnesota Department of Natural Resources (DNR), M. Marron and R. L. Benjamin, Wisconsin, DNR, pers. comm.), we suggest that resource managers determine the summer and winter ranges of *I. punctatus* in the St. Croix River, and evaluate the impact of *I. punctatus* on both glochidial mortality and availability of hosts for *Q. fragosa*.

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